Letter to the Editor

Pyrazinamide Is Not Effective against Intracellularly Growing *Mycobacterium tuberculosis*

In in vitro assays, the antibiotic pyrazinamide (PZA) is active against *Mycobacterium tuberculosis* in acidic medium (6), and intracellular activation of this drug in acidic loci of phagocytes (5) has long been proposed. However recent studies on PZA action on intracellular tubercle bacilli (2, 3) could establish only a bacteriostatic activity of the drug. Moreover, in one study PZA was added right from the beginning of phagocytosis (2), and consequently the bacilli could not undergo active multiplication before drug addition. Because we recently developed a J-774 murine macrophage cell line model for intracellular growth of mycobacteria (4), we decided to investigate the action of PZA on tubercle bacilli growing actively inside J-774 macrophages.

M. tuberculosis $H_{37}Rv$ was grown in complete 7H9 medium (Difco Laboratories) containing 0.05% (vol/vol) Tween 80. Exponentially growing bacteria (optical density of 0.15 at 650 nm) were used to infect the macrophages for 4 h, and then their intracellular growth was established as reported earlier (4). Control experiments showed that bacilli did not grow significantly in the RPMI culture medium during the time alloted for these experiments (7 days).

In one set of experiments, PZA (25, 50, or 100 μg/ml) was added after 48 h of intracellular growth of bacteria, and bacterial viability was measured after 2 and 5 days of drug addition on 7H10 agar medium (Difco), as reported earlier (4).

In a second set of experiments, to avoid any eventual inhibition of drug penetration in already infected macrophages, we pretreated the macrophages with PZA (50 or 100 μg/ml) before phagocytosis. Since PZA penetration is almost complete within 3 h in mouse macrophages (1), we decided on a 4-h pretreatment. In this set of experiments, the drug was present throughout the course of infection and multiplication and was renewed at days 2 and 4.

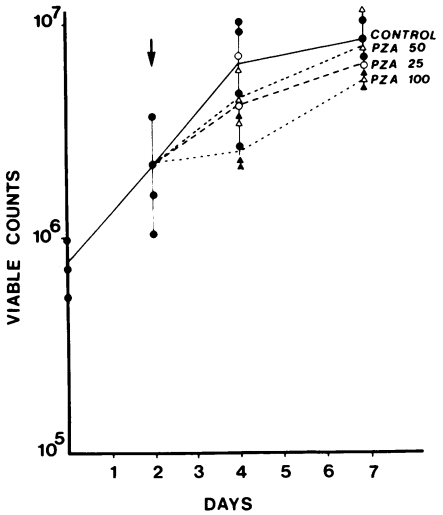

FIG. 1. Action of PZA against intracellularly multiplying *M. tuberculosis*. Bacteria were phagocytized during 4 h and allowed to multiply inside J-774 macrophages for 2 days. PZA at 25, 50, and 100 μg/ml was added at day 2, and viable counts were determined at days 4 and 7. The arrow shows the time of drug addition. Symbols: ●, control; ○, PZA at 25 μg/ml; △, PZA at 50 μg/ml; ▲, PZA at 100 μg/ml.

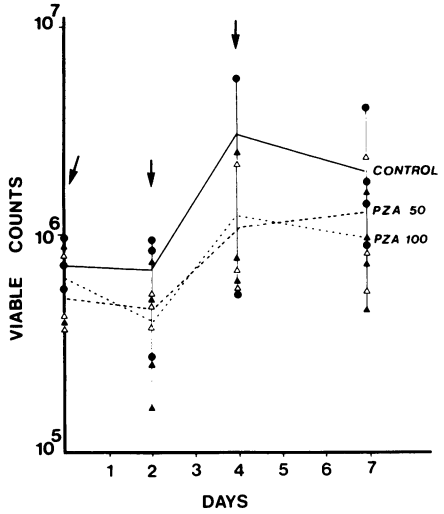

FIG. 2. Action of PZA on *M. tuberculosis* growing inside PZA-pretreated macrophages. J-774 cells were pretreated with either 50 or 100 μg of PZA per ml before phagocytosis of tubercle bacilli. Corresponding concentrations of PZA were present throughout the infection and subsequent multiplication steps. The arrows indicate the times of drug addition. Symbols: ●, control; △, PZA at 50 μg/ml; ▲, PZA at 100 μg/ml.

Our results obtained with three different experiments each are illustrated in Fig. 1 and 2. PZA was not active in the first set of experiments (Fig. 1) and barely bacteriostatic in macrophages pretreated with the drug before infection (Fig. 2). It should, however, be noted that Fig. 1 represents an in vitro analogy to a clinical situation, in which chemotherapy starts after extensive growth of the tubercle bacilli in the host. Consequently, our data question the selective activation of PZA in intracellular loci.

LITERATURE CITED

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